

Study on Therapeutic Management of Tick-Borne Diseases in Cross-Bred Cattle

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Abstract

Tick-borne hemoprotozoan diseases such as Anaplasmosis and Theileriosis, caused by obligate intra-erythrocytic parasites result in significant economic losses for the livestock industry worldwide. In the current study, 40 cross-bred cattle with tick infestations were examined showing clinical signs such as weakness, inappetence, pyrexia, pale mucous membranes, anemia, lymph node swelling, and nasal discharge. Haematological analysis revealed anaemia, low erythrocyte count, and decreased packed cell volume. Peripheral thin blood smear showed the presence of *Anaplasma* spp. (03/40), *Theileria* spp. (08/40), and co-infection of *Theileria* spp. with *Anaplasma* spp. (08/40) in erythrocytes as well as Koch's Blue Bodies in lymphocytes. On the first day, cases of anaplasmosis (n=03) were treated with Oxytetracycline Long acting @ 22 mg/kg b.wt. intramuscularly, theileriosis cases (n=08) were treated with Inj. Buparvaquone @ 2.5 mg/kg b.wt. intramuscularly and mixed infections (n=08) were treated with both drugs. All clinical cases also received symptomatic treatment such as Flunixin meglumine @ 1.1 - 2.2 mg/kg b.wt. and liver tonic (Inj. Rumeric 10 ml). On the seventh day only two animals showed theileria infection and were treated again with Buparvaquone. It can be concluded that 100% of bovine anaplasmosis cases recovered with one dose of oxytetracycline, while 87.5% of bovine tropical theileriosis cases recovered after one dose of buparvaquone, and 100% of cases recovered after two doses of Buparvaquone. Anaplasmosis and Theileriosis co-infections were prevalent in the cattle of this region and can be successfully treated with the above therapeutic management.

Keywords: Anaplasmosis, cattle, Buparvaquone, Giemsa stain, Oxytetracyclin, Theileriosis.

Introduction

Anaplasmosis and theileriosis are tick-borne diseases caused by obligate intra-erythrocytic parasites. Both diseases are considered among the ten most economically important parasitic diseases affecting ruminants in India and have a significant impact on the global livestock industry (Ganguly *et al.*, 2017; Kumar *et al.*, 2023a). According to the 20th livestock census, there is an annual cumulative loss of 46199.31 million rupees recorded as loss in milk production, treatment expenses, and leather due to tick infestations. Additionally, there are annual losses of 14877.15 million rupees due to tick-borne diseases, resulting in a total loss of 61076.46 million rupees. These diseases are mainly transmitted by Ixodid ticks of *Rhipicephalus* spp. and *Hyalomma* spp. (Ali *et al.*, 2012). The monsoon season favors the breeding of these ticks leading to a rise in tick population and prevalence during this time (Vahora *et al.*, 2012). Cattle experience stress, immunodepression, hypersensitivity, depreciation in hide quality, progressive weight loss, and toxicosis due to tick infestation (Lorusso *et al.*, 2013). Cross-bred cattle are more susceptible to these diseases compared to indigenous cattle (Kaur *et al.*, 2021). Clinical signs include high fever, weakness, inappetence, enlarged lymph nodes, and anemia; later stages may show lacrimation, corneal opacity, nasal discharge and diarrhea (Radostits *et al.*, 2007; Kumar *et al.*, 2023b). Diagnostics include direct microscopic examination, serological analysis, and molecular detection.

An inexpensive direct microscopic examination is commonly used in field studies to analyze a large number of suspected cases, detecting piroplasm and schizonts in smears made from whole blood and lymph node aspirates stained with Geimsa's stain (Aktas *et al.*, 2006). This study described the clinical manifestations, diagnosis, and therapeutic management of co-infections of Theileriosis and Anaplasmosis in cross-bred cattle with a history of tick infestations.

Materials and Methods

History and Clinical Observations

The study was conducted at Kanha Upvan Gaushala, Partapur, Meerut, in October 2023. A total of 40 cross-bred cattle of different age groups, infested with ticks and showing clinical signs such as weakness, lack of appetite, pyrexia, pale mucous membranes, anemia, lymph node swelling, and nasal discharge, were selected and screened for hemoprotozoan infections. Blood was collected aseptically from each of the 40 cross-bred cattle using labeled sterile EDTA vacutainers (BD, USA) and transported to the laboratory on ice for microscopic examination.

Laboratory Examination

Peripheral thin blood smears were prepared according to the standard protocol, fixed, and stained with Giemsa stain. A smear was observed under an oil immersion lens at 100 X magnification for the detection of hemoprotozoa (Potgieter *et al.*, 2004). The whole blood collected in EDTA at 1 mg/ml of blood was also processed for hematological parameters hemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC), PCV (packed cell volume), and platelets (Schalm *et al.*, 1975).

Treatment

Initially, on day 0, the forty cross-bred cattle were screened based on tick infestation and clinical manifestations. Approximately 3–4 ml of peripheral blood was collected aseptically in EDTA vials, and a thin blood smear was prepared, stained with Giemsa stain, and observed under an oil immersion lens for the detection of hemoprotozoa (Potgieter *et al.*, 2004). Based on microscopic examination on day 1, Anaplasma positive cases were treated with Oxytetracycline-LA at a dose rate of 22 mg/kg b.wt. intramuscularly along with supportive treatment using injection Flunixin meglumine at 1.1–2.2 mg/kg b.wt. and injection liver tonic at 1 ml/40 kg intramuscularly. Theileria positive cases were treated with injection Buparvaquone at a dose rate of 2.5 mg/kg b.wt. intramuscularly with similar supportive treatment for Anaplasma positive cases. Cattle with co-infection (Anaplasma + Theileria) were treated with a combination of Oxytetracycline-LA at 22 mg/kg b.wt. and Buparvaquone at 2.5 mg/kg b.wt. intramuscularly, along with supportive treatment. After one week, the blood samples from the affected cattle were screened again for parasitemia to assess the treatment's effectiveness.

Result and Discussion

A thin Giemsa stained blood smear examination revealed the presence of *Anaplasma* spp. (03/40), *Theileria* spp. (08/40), and co-infection of *Theileria* spp. with *Anaplasma* spp. positive (08/40) parasites in red blood corpuscles, along with the presence of Koch's Blue Bodies in lymphocytes (Fig. 1-3). *A. marginale* piroplasm is blue-perpule in color, smaller (0.3–1 μ m), and round or oval in shape. *T. annulata* has a smaller (0.5–1.5 μ m) piroplasm that is shaped like an oval, spherical, ovoid, or maltese cross. It is typical for each erythrocyte to contain a single organism; however, other forms have also been reported. Out of 19 animals suffering from parasitemia, only two were found positive for Theileriosis after one week of treatment, and the same treatment was repeated again on day one. After 14 days, all cattle were recovered completely, and no parasite was detected in a thin blood smear examination.



Figure 1: Geimsa-stained blood smears revealed the presence of dense, small, round, or oval-shaped *A. marginale* organisms in the erythrocytes of the blood smear.

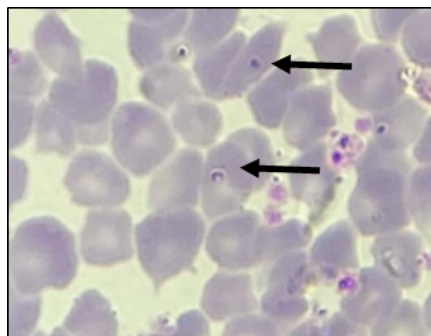


Figure 2: Geimsa stained blood smears revealed the presence of small, rod-shaped, or oval spherical, ovoid, or maltese cross shape *T. annulata* organism in the erythrocytes of blood smear.

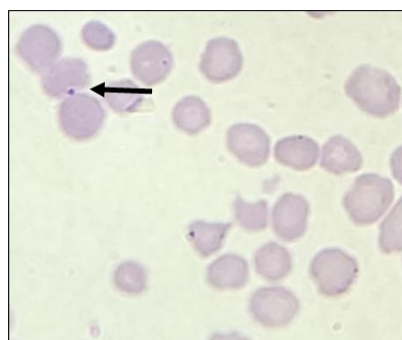
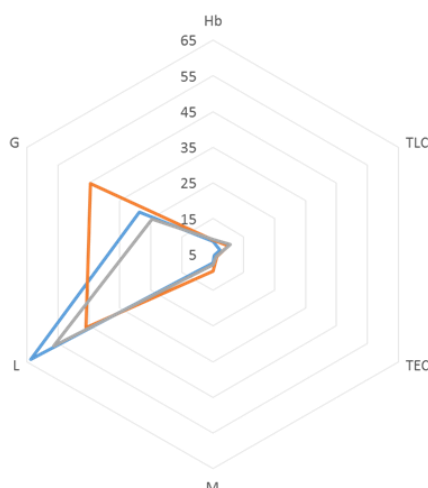


Figure 3: Geimsa stained blood smears revealed the presence of dense, small, round ,or oval-shaped *A. marginale* and small, rod-shaped, or oval spherical, ovoid *T. annulata* organism in the erythrocytes of blood smear.

Table 1: Haematological parameters of cattle affected with Anaplasma, Theileria and co-infection of Anaplasma and Theileria infection.

S.No.	Parameter	Reference value	Mean \pm S.E. (Lower bound – Upper bound at 95% confidence interval)			F value (P value)
			<i>Anaplasma marginale</i>	<i>Theileria annulata</i>	<i>Anaplasma marginale</i> & <i>Theileria annulata</i>	
1	Hb (g/dL)	8.0-15.0	7.3 \pm 0.62 (5.75 - 8.85)	7.36 \pm 1.22 (6.34 - 8.38)	8.2 \pm 1.59 (6.87 - 9.53)	0.94 (0.41)
2	TEC ($\times 10^6/\mu\text{L}$)	5.0-10.0	6.33 \pm 0.51 (5.06 - 7.61)	4.95 \pm 1.07 (4.06 - 5.84)	4.78 \pm 0.81 (4.1 - 5.45)	3.41 (0.06)
3	TLC ($\times 10^3/\mu\text{L}$)	4.0-12.0	13.13 ^a \pm 0.45 (12.01 - 14.25)	12.55 ^a \pm 2.08 (10.81 - 14.29)	15.64 ^b \pm 1.45 (14.42 - 16.85)	7.12 (0.01)
4	Neutrophil (%)	15.0-45.0	47.1 \pm 1.71 (42.86 - 51.34)	43.69 \pm 7.23 (37.65 - 49.73)	47.26 \pm 1.42 (46.08 - 48.45)	1.2 (0.33)
5	Lymphocyte (%)	45.0-75.0	45.87 ^a \pm 5.39 (32.47 - 59.27)	61.29 ^b \pm 10.81 (52.25 - 70.33)	63.75 ^b \pm 9.02 (56.21 - 71.29)	4.01 (0.04)
6	Eosinophil (%)	0.0-20.0	4.37 \pm 0.51 (3.09 - 5.64)	4.23 \pm 0.47 (3.83 - 4.62)	4.35 \pm 0.37 (4.04 - 4.66)	0.21 (0.81)
7	Monocyte (%)	2.0-7.0	6.57 \pm 0.31 (5.81 - 7.33)	7.31 \pm 1.62 (5.95 - 8.67)	6.74 \pm 1.73 (5.29 - 8.19)	0.37 (0.69)
8	PCV (%)	24.0-46.0	27.47 \pm 0.91 (25.21 - 29.72)	26.6 \pm 2.21 (24.76 - 28.44)	27.73 \pm 0.68 (27.16 - 28.29)	1.09 (0.36)
9	Platelets	100-800	305.2 ^a \pm 5.96 (290.39 - 320.01)	318.35 ^b \pm 7.35 (312.2 - 324.5)	321.74 ^b \pm 6.62 (316.21 - 327.27)	6.38 (0.01)
10	Relative parasitaemia (%)		68.33 \pm 3.51 (59.61 - 77.06)	69.63 \pm 4.6 (65.78 - 73.47)	68 \pm 6.44 (62.62 - 73.38)	0.19 (0.83)

#Reference range adopted from Schalm et al., 1975. Hb=Hemoglobin, TEC=Total erythrocyte count, TLC=Total leukocyte count, PCV=Packed cell volume, SEM=Standard error of mean, Means bearing different superscript along a row differed significantly at $p \leq 0.05$.



COMPARATIVE HAEMATOLOGY

— Theileria — Anaplasma — Co-infection

Theileriosis causes more depression in haemoglobin and total erythrocyte count, than Anaplasmosis or mixed infection thereof, in female cross-bred cattles.

Mixed infection of *Anaplasma* and *Theileria* causes greater rise in total leukocyte count than Theileriosis or Anaplasmosis infection alone, in female cross-bred cattles.

The haematology of bovine Anaplasmosis, Theileriosis and co-infection of Anaplasmosis and Theileriosis (Table 1) revealed hemolytic anemia (normocytic hypochromic anemia) indicating the destruction of erythrocytes by

Anaplasma spp. and *Theileria* spp. and activation of the haematopoietic system in response to erythrophagocytosis (Kumar and Sangwan, 2010; Chandrate *et al.*, 2018). Anemia is a significant hematological abnormality in bovine anaplasmosis, theileriosis, and co-infection of both diseases causing thinning of the blood, a light coloring of the mucous membrane, and eventually a yellowish discoloration of the mucosa due to erythrocyte destruction (Constable *et al.*, 2017). According to DeVos *et al.* (2006), De *et al.* (2012), and Khaki *et al.* (2018), the anemia may be caused by oxidative stress, complement system activation, immune-mediated erythrocyte destruction, and widespread erythrophagocytosis by *Anaplasma* and *Theileria*. Jaundice can also result from a large-scale destruction of red blood cells and the release of their contents into the blood (Jaswal *et al.*, 2015). In cases of bovine Anaplasmosis, the quick destruction of erythrocytes by phagocytic cells may lead to the release of immature red blood cells from the bone marrow, potentially leading to regenerative anemia (Subramanian *et al.*, 2018). Leukocytosis with neutrophilia was observed in the leukogram of cattle infected with Theileriosis, Anaplasmosis, and co-infection of both illnesses. The observed increase in neutrophil counts in bovine cases of Anaplasmosis, Theileriosis, and co-infection with both diseases was anticipated and may be related to the deleterious impact of toxins or metabolites from *Anaplasma* and *Theileria* species on hemopoietic organs, including the bone marrow, as well as their disruption of leukogenesis. Chronic infection linked to *Theileria* and *Anaplasma* species may also explain the leukocytosis observed in animals infected with these pathogens.

The preferred medication for treating bovine Anaplasmosis, is Oxytetracycline. After only one injection dose, 100% of cases recovered without experiencing any negative side effects. Because of its method of action, which involves inhibiting protein synthesis, this medication exhibits rickettsiostatic effects (Scholar and Pratt, 2000). Coetzee *et al.* (2009) found that the majority (70%) of *Anaplasma marginale* inclusion bodies exhibited chromatin condensation and vacuolization following Oxytetracycline therapy, based on electron microscopy findings. The choice of treatment for bovine tropical Theileriosis is Buparvaquone. In the current investigation, Buparvaquone recovered 87.5% of cases, while two injections of Buparvaquone one week apart resulted in full recovery with no adverse effects. Cattle affected by co-infection of *Anaplasma* spp. and *Theileria* spp. and treated with a combination of Oxytetracycline and Buparvaquone at the same dose rate found 100% recovery with only a single dose and no adverse effects. The results are consistent with the 55% efficacy reported by Nasir (2000). On the other hand, Muhammad *et al.* (1999) reported a 93% effectiveness rate for both Oxytetracycline and Buparvaquone in Faisalabad. This discrepancy can be attributed to the combination of the two medications. In contrast, Patil *et al.* (1995) claimed an 80% cure rate in India, and Anonymous (1993) reported a 98.8% cure rate.

Conclusion

In the current investigation, animals with bovine Anaplasmosis treated by Oxytetracycline-LA injections at a dose rate of 22 mg/kg b.wt. showed tolerance to the treatment, and 100% of cases recovered after just a single dose. One injection of Buparvaquone-furosemide at a dose rate of 2.5 mg/kg b.wt. resulted in the recovery of 87.5% of Theileriosis cases, whereas two injections of Buparvaquone-furosemide, spaced 1 week apart, resulted in the recovery of 100% of cases. Co-infection of *Theileria* spp. and *Anaplasma* spp. in the cattle treated with a cocktail of Oxytetracycline and Buparvaquone at the same dose rate found 100% recovery only with a single dose. It may be concluded that the co-infection of theileriosis and anaplasmosis was common in the Gashalas cattle and that it can be successfully treated with the current therapeutic treatment without causing any negative side effects.

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Contribution by Authors

Equal contribution. All authors declared that 'written informed' consent was obtained from the approved parties for the publication of this article and accompanying images.

Conflict of Interests

There is no conflict of interest.

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